

# PANEL #3 STRUCTURAL AND FUNCTIONAL ENDPOINTS

## Corneal Confocal Microscopy in Peripheral nerve disease

Michael Polydefkis, MD MHS

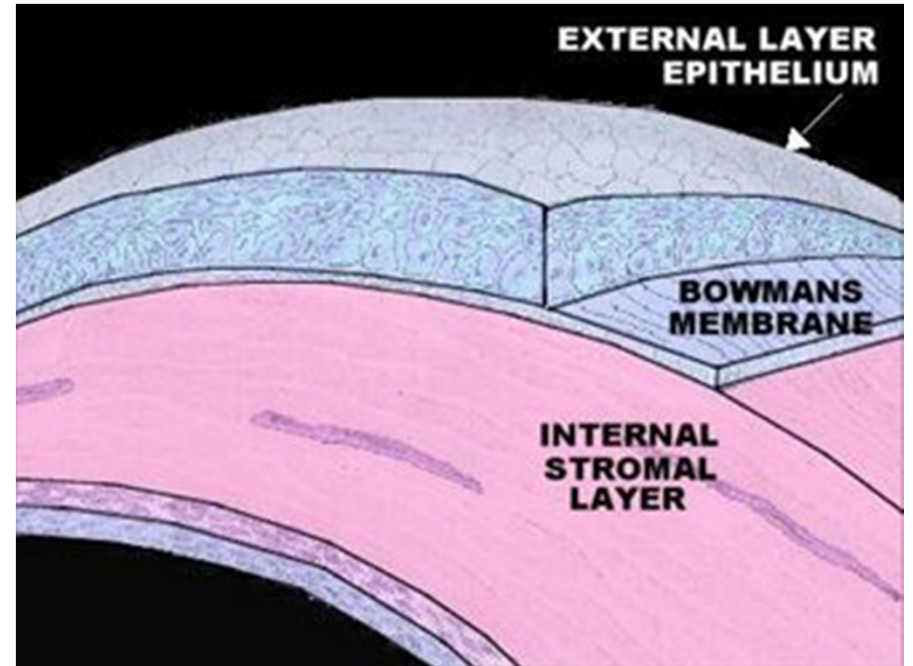
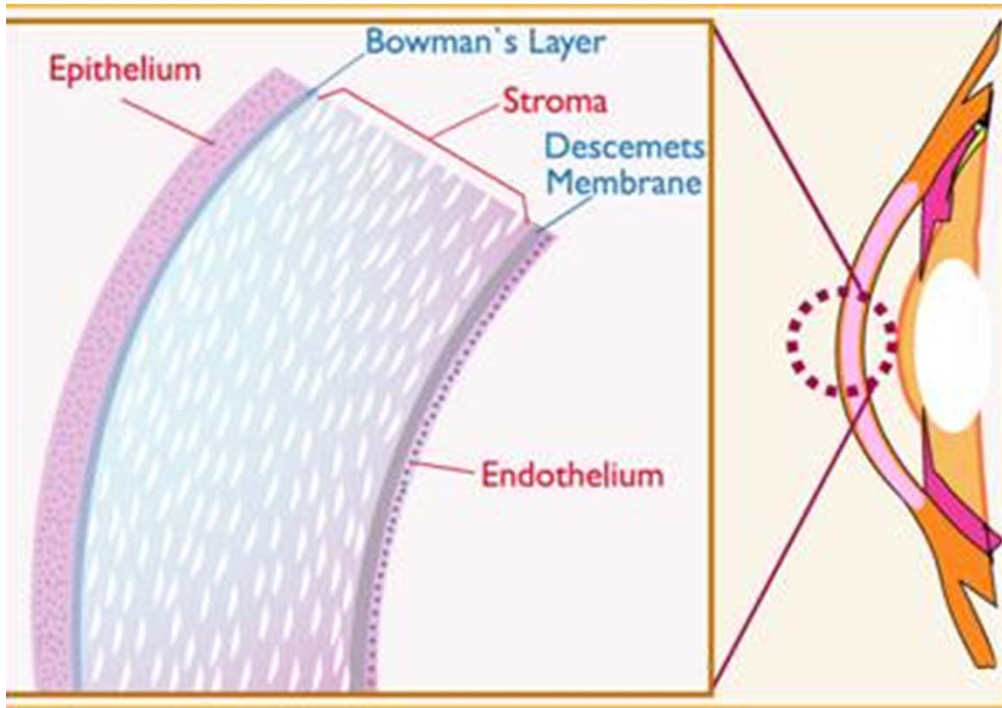
Associate Professor

Johns Hopkins Neurology

# Disclosures

- I am a neurologist
  - See patients with PN disease
  - Direct a NCV/EMG laboratory
  - Direct a Cutaneous Nerve Laboratory
- I am not an expert in confocal microscopy
  - Two afternoons observing confocal microscopy at Wilmer Eye Institute

# Anatomy



## Bowman's layer

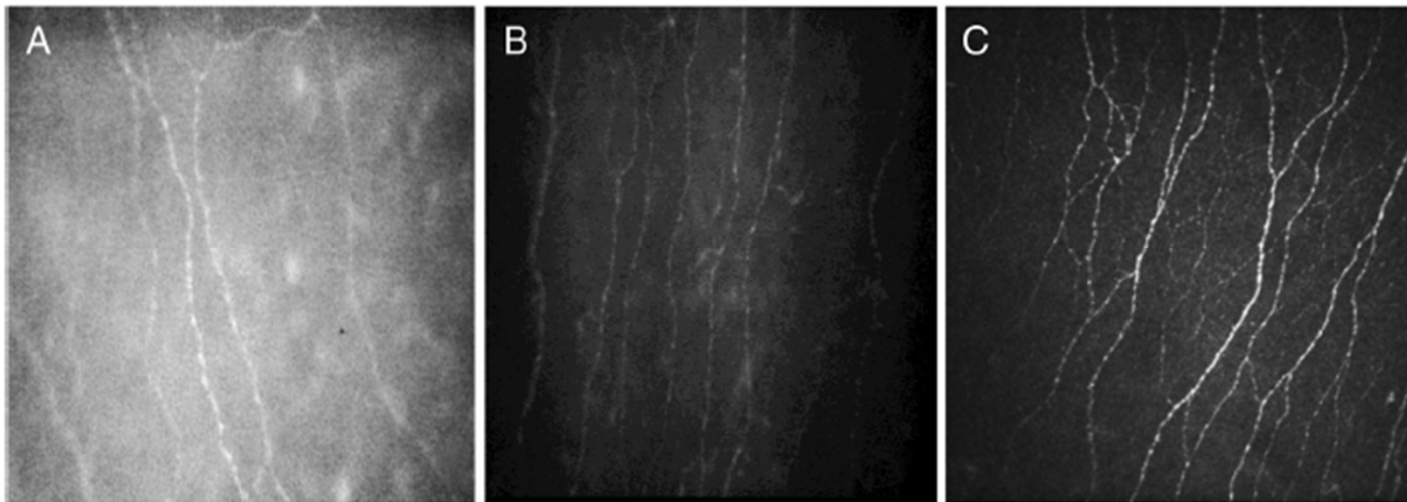
- Acellular
- Composed of fine collagen fibrils - arranged in random distribution
- ~10  $\mu\text{m}$  thick
- Is limited anteriorly by the basement membrane of the corneal epithelium.
- Most are sensory

# Corneal Nerve

- Most of the nerve fibres are sensory in function and originate from the ophthalmic division of the trigeminal nerve; however, there is a small peri-limbal sympathetic nerve plexus, presumably derived from the superior cervical ganglion.

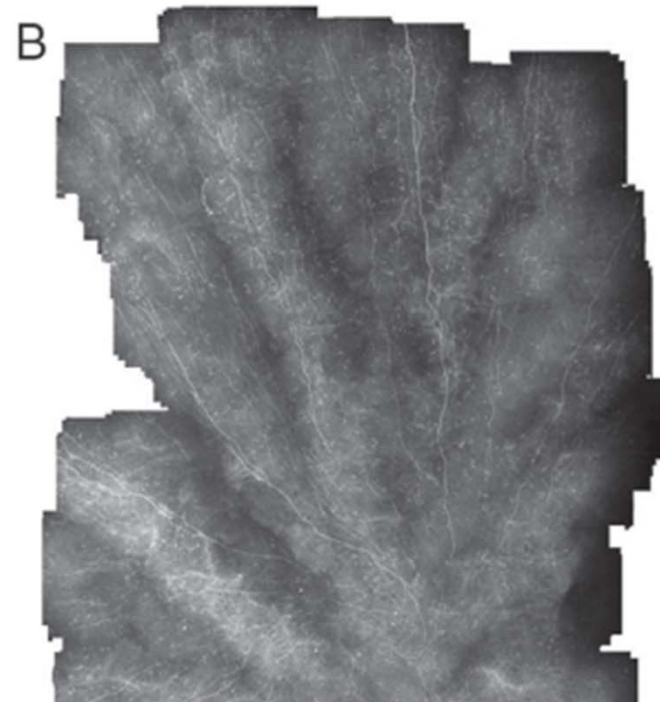
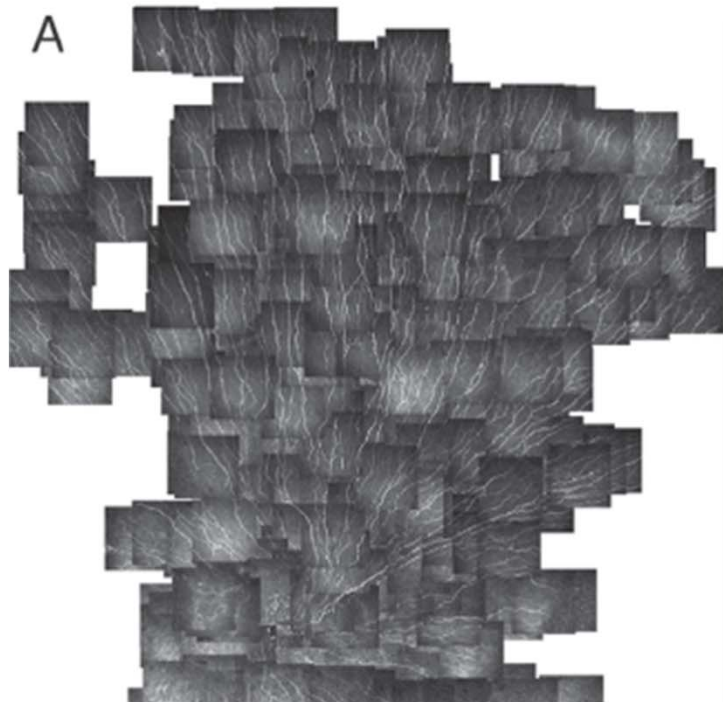
# Different forms of confocal microscopy

- Tandem scanning confocal microscope (A)
- Slit scanning confocal microscope (Nidek Confoscan 4, Nidek Technologies, Padova, Italy and Tomey Confoscan P4, Tomey, Erlangen, Germany) – (B)
- Laser scanning confocal microscope, namely the Rostock Corneal Module (HRT III RCM) of the Heidelberg Retina Tomograph III (Heidelberg GmbH, Heidelberg, Germany) – (C)



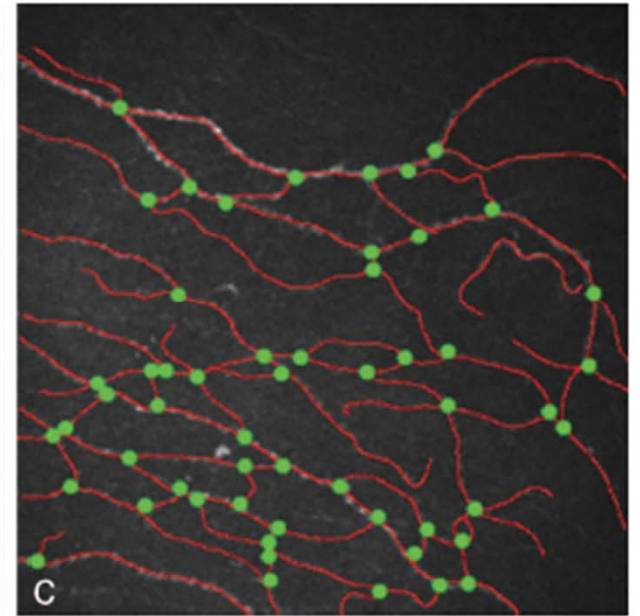
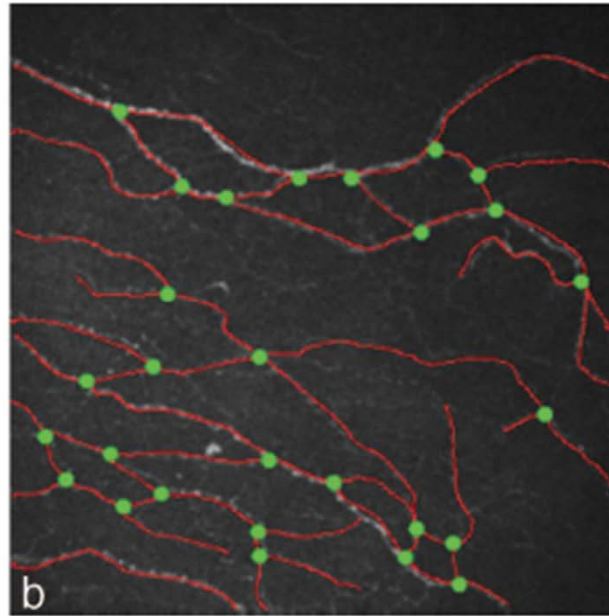
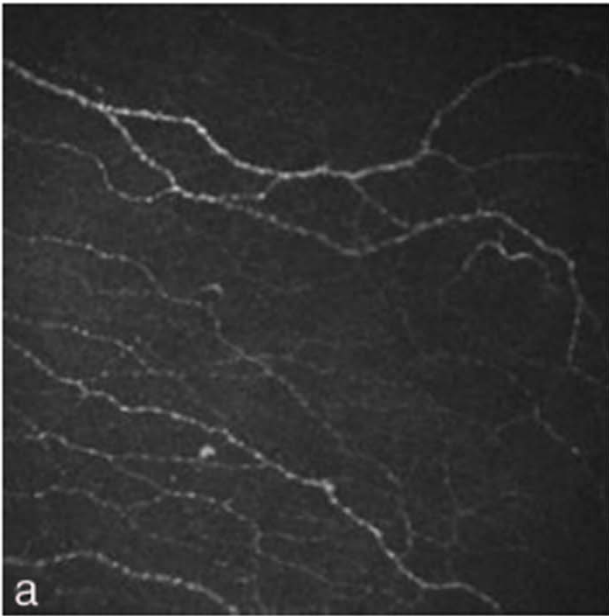
# Differences in techniques

- Different methods have been used
  - number of images selected and analyzed per subject
  - sampling method
  - Metric: nerves/mm<sup>3</sup>, nerves/image
  - Different assessments: NFD, NFL, NFBD



# Reproducibility of CNFD

- CCM images have used manual delineation of the nerve fibers by experts

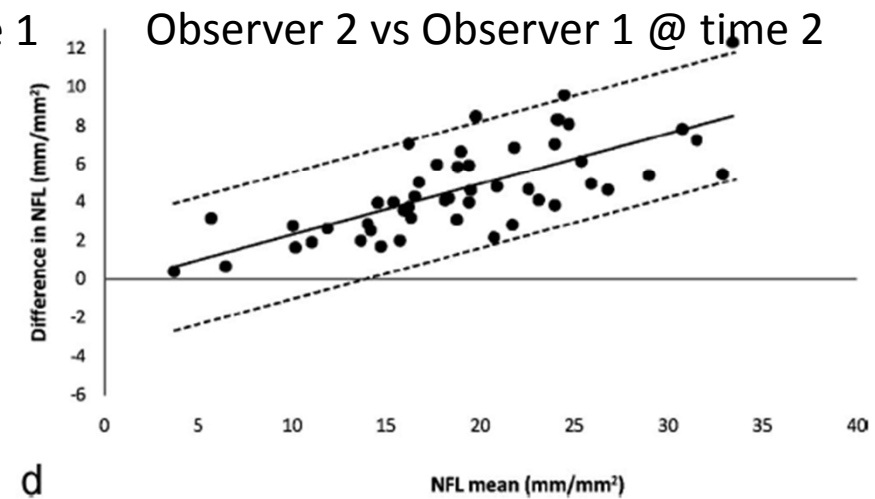
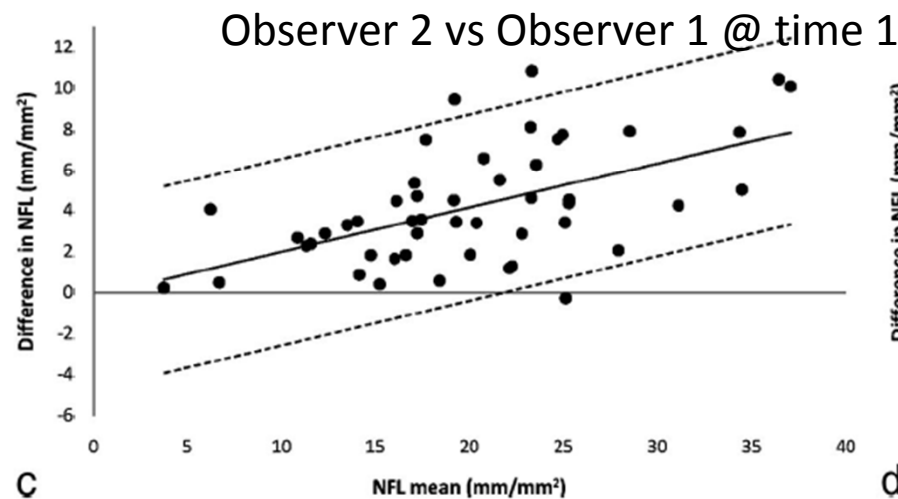
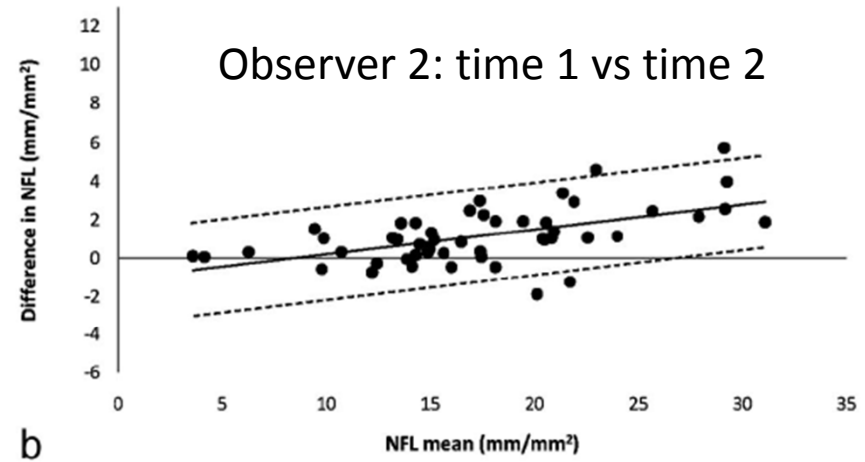
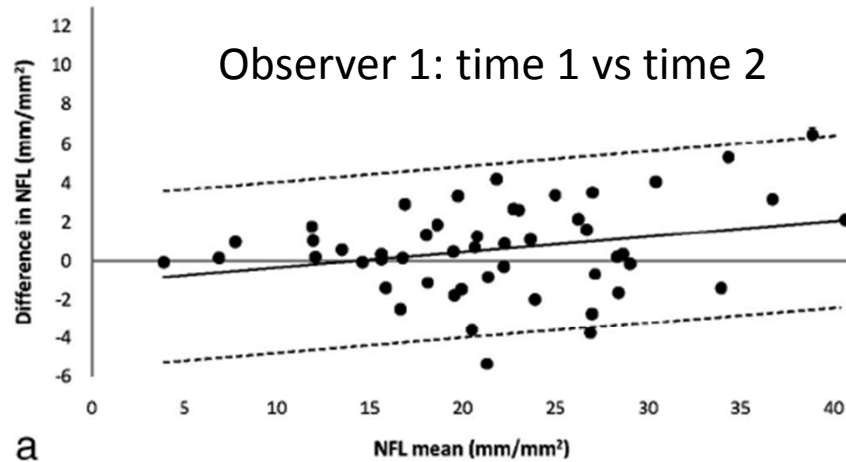


# Repeatability of Measuring Corneal Subbasal Nerve Fiber Length in Individuals With Type 2 Diabetes

*Nathan Efron, D.Sc., Katie Edwards, Ph.D., Nicola Roper, B.A.(Oxon.), Nicola Pritchard, B.App.Sc.(Optom), Geoff P. Sampson, Ph.D., Ayda M. Shahidi, B.Sc.(Optom.), Dimitrios Vagenas, Ph.D., Anthony Russell, Ph.D., Jim Graham, Ph.D., Mohammad A. Dabbah, Ph.D., and Rayaz A. Malik, Ph.D.*

- Images were captured from the corneas of 50 subjects with type 2 diabetes mellitus who showed varying severity of neuropathy, using the Heidelberg Retina Tomograph 3 with Rostock Corneal Module.
- Semi-automated nerve analysis software was independently used by two observers to determine NFL from images of the subbasal nerve plexus. This procedure was undertaken on two occasions, 3 days apart.
- At least eight images of the subbasal nerve plexus were captured.
- The first image taken from each subject was analyzed by both readers

# Intra and inter-rater repeatability



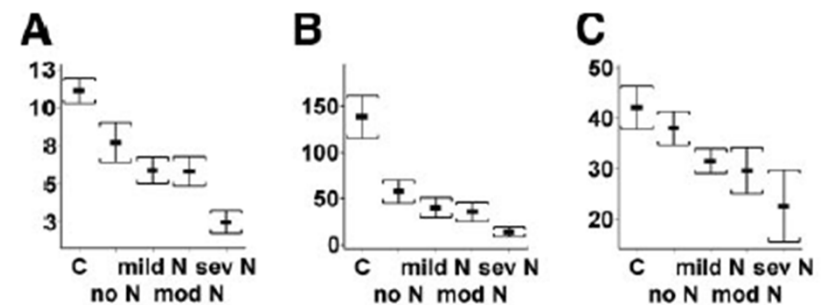
Correlation of one reader counting the same image twice: 0.95 (95% CI 0.92– 0.97)

Correlation of two readers counting the same image: 0.95 (95% CIs: 0.74 –1.00)

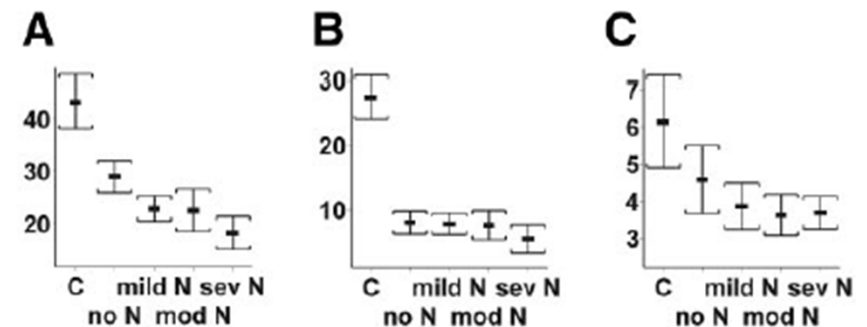
# CNFD correlates with other peripheral nerve measures

- 54 DM subjects of varying degrees of DPN (NDS), and 15 control subjects
  - DM subjects: no PN = 10, mild DPN = 18, moderate DPN=15, and severe DPN=11
- IENFD  $r_s = 0.385$ ,  $p=0.001$
- Sural SNAP  $r_s = 0.176$ ,  $p$  NS
- PNCV  $r_s = 0.250$ ,  $p$  NS
- CDT  $r_s = -0.399$ ,  $p=0.003$
- HP-VAS(0.5)  $r_s = -0.291$ ,  $p=0.04$
- DB-HRV  $r_s = 0.348$ ,  $p=0.02$

IEINF



CNF



density

length

branch density

# Original Article

## Surrogate Markers of Small Fiber Damage in Human Diabetic Neuropathy

Cristian Quattrini,<sup>1</sup> Mitra Tavakoli,<sup>1</sup> Maria Jeziorska,<sup>2</sup> Panagiotis Kallinikos,<sup>1</sup> Solomon Tesfaye,<sup>3</sup> Joanne Finnigan,<sup>4</sup> Andrew Marshall,<sup>4</sup> Andrew J.M. Boulton,<sup>1</sup> Nathan Efron,<sup>5</sup> and Rayaz A. Malik<sup>1</sup>

	IENFD (no/mm)	IENFBD (no/mm <sup>2</sup> )	IENFL (μm)	CNFD (no/mm <sup>2</sup> )	CNFBD (no/mm <sup>2</sup> )	CNFL (mm/mm <sup>2</sup> )
NDS (0–10)	−0.425 0.001	−0.376 0.006	−0.343 0.012	−0.299 0.028	−0.107 NS	−0.088 NS
SNOL (ms)	0.011 NS	0.092 NS	0.086 NS	−0.003 NS	0.459 0.002	0.056 NS
SNAP (μV)	0.351 0.015	0.394 0.007	0.295 0.047	0.176 NS	−0.114 NS	−0.018 NS
SNCV (m/s)	0.246 NS	0.286 0.054	0.333 0.024	0.176 NS	−0.075 NS	0.083 NS
PNOL (ms)	−0.147 NS	−0.340 0.018	−0.259 NS	−0.035 NS	0.072 NS	−0.070 NS
PNAP (mV)	0.242 NS	0.219 NS	0.315 0.027	0.084 NS	−0.072 NS	−0.070 NS
PNCV (m/s)	0.406 0.003	0.391 0.005	0.511 <0.001	0.250 NS	−0.200 NS	0.181 NS
PNFL (ms)	−0.364 0.032	−0.160 NS	−0.029 NS	0.016 NS	0.272 NS	−0.130 NS
TNOL (ms)	−0.406 0.004	−0.324 0.025	−0.242 NS	−0.144 NS	0.130 NS	−0.001 NS
TNAP (mV)	0.202 NS	0.269 NS	0.196 NS	0.259 NS	0.027 NS	0.219 NS
TNCV (m/s)	0.370 0.014	0.225 NS	0.221 NS	0.127 NS	−0.236 NS	0.193 NS
TNFL (ms)	−0.589 < 0.001	−0.473 0.005	−0.128 NS	−0.035 NS	0.313 NS	−0.017 NS
CDT (percentile)	−0.466 < 0.001	−0.408 0.003	−0.285 0.041	−0.399 0.003	−0.025 NS	−0.081 NS
HP-VAS 0.5 (percentile)	−0.311 0.028	−0.297 0.039	−0.223 NS	−0.291 0.040	−0.269 NS	−0.134 NS
HP-VAS 5.0 (percentile)	−0.357 0.010	−0.220 NS	−0.146 NS	−0.264 NS	−0.267 NS	−0.221 NS
HP-VAS 0.5–5.0 (percentile)	0.009 NS	0.255 NS	0.148 NS	0.032 NS	0.029 NS	−0.069 NS
DB-HRV (percentile)	0.268 NS	0.204 NS	0.148 NS	0.348 0.024	0.073 NS	0.202 NS

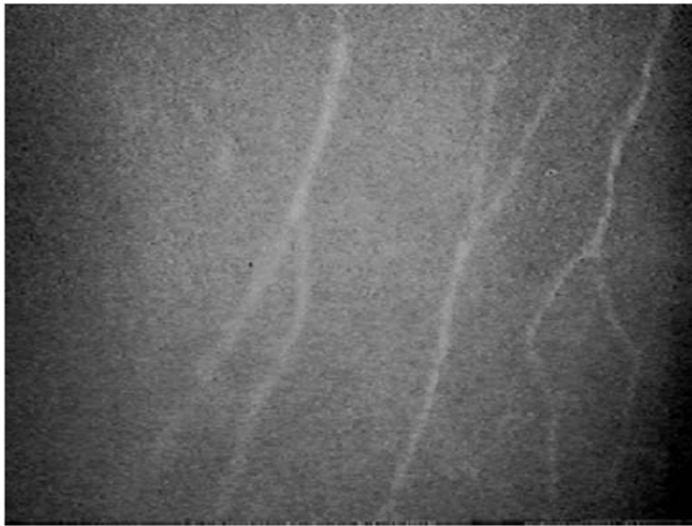
# Corneal innervation and sensation

- Reduced corneal sensitivity was associated with reduced vibration perception, suggesting a link with diabetic peripheral neuropathy. Nielsen NV. *Acta Ophthalmol* 1978; 56: 406–411.
- Corneal sensation, as measured by non-contact corneal aesthesiometer – sensation to a brief puff of air through a bore 0.5 mm in diameter onto the center of the cornea is measured
  - Reduced in a number of neuropathy conditions including CMT, Fabry's disease and diabetes.

# Corneal confocal microscopy: a non-invasive surrogate of nerve fibre damage and repair in diabetic patients

R. A. Malik<sup>1</sup>, P. Kallinikos<sup>2</sup>, C.A. Abbott<sup>1</sup>, C.H.M. van Schie<sup>1</sup>, P. Morgan<sup>2</sup>, N. Efron<sup>2</sup>, A. J. M. Boulton<sup>1</sup>

Control



DM - Severe



Parameter	Control (n=18)	Mild (n=4)	Moderate (n=7)	Severe (n=7)
Age (yrs.)	57.8±11.5	53.0±18.5	60.1±7.4	58.3±12.4
Diabetes duration (yrs.)	0	21.3±3.6	20.8±5.1	26.0±7.4
Diabetes (Type 1/Type 2)		2/2	2/5	3/4
HbA <sub>1c</sub> (%)	<6.5	7.8±0.8	8.1±1.2	8.2±1.4
Parameter	Control (n=18)	Mild (n=4)	Moderate (n=7)	Severe (n=7)
NDS	0	1.2±0.6	3.5±0.9	7.5±1.2
PMNCV (ms <sup>-1</sup> )	>45	37.6±3.4	33.5±4.2	26.2±4.5
VPT (volts)	<14	11.2±4.3	37.0±6.7	48.1±5.5
TPT (JND)	<15	17.6±2.2	23.8±1.1	>25.0

# Corneal confocal microscopy: a non-invasive surrogate of nerve fibre damage and repair in diabetic patients

R. A. Malik<sup>1</sup>, P. Kallinikos<sup>2</sup>, C.A. Abbott<sup>1</sup>, C.H.M. van Schie<sup>1</sup>, P. Morgan<sup>2</sup>, N. Efron<sup>2</sup>, A. J. M. Boulton<sup>1</sup>

- One eye selected at random.
- Several scans of the entire depth of the cornea were recorded to acquire satisfactory images of all corneal layers.
- 3-5 high quality images of Bowman's layer.
- The investigator who examined the cornea with the confocal microscope and who undertook morphometric measurements was blinded with respect to the neuropathy severity in DM patients.
  - (i) Nerve fiber density (NFD)—total number of major nerves/mm<sup>2</sup> corneal tissue
  - (ii) Nerve fiber length (NFL)—total length of all nerve fibers and branches (mm per mm<sup>3</sup>)
  - (iii) Nerve branch density (NBD)
- To estimate the error in NFD, NFL and NBD, images were acquired and analyzed two occasions separated by at least 48h for 15 subjects.
  - The coefficient of variation of these parameters was:
    - 12% for NFD,
    - 9% for NFL
    - 24% for NBD
- Three months after the first measurement, repeatability was tested by reanalyzing 24 (20%) randomly selected frames.
  - The interobserver repeatability was:
    - 93% for NFD,
    - 91% for diameter measurement and
    - 87% for beading counts.

Parameter	Control (n=18)	Mild (n=4)	Moderate (n=7)	Severe (n=7)
NFD (number/mm <sup>2</sup> )	44.5±14.1	37.2±4.6	24.4±5.5	21.2±9.4
NFL (mm/mm <sup>2</sup> )	13.5±0.3	10.8±0.9	7.5±1.1	4.3±1.5
NBD (number/mm <sup>2</sup> )	78.9±30.4	32.3±9.04	29.0±8.8	20.2±22.2

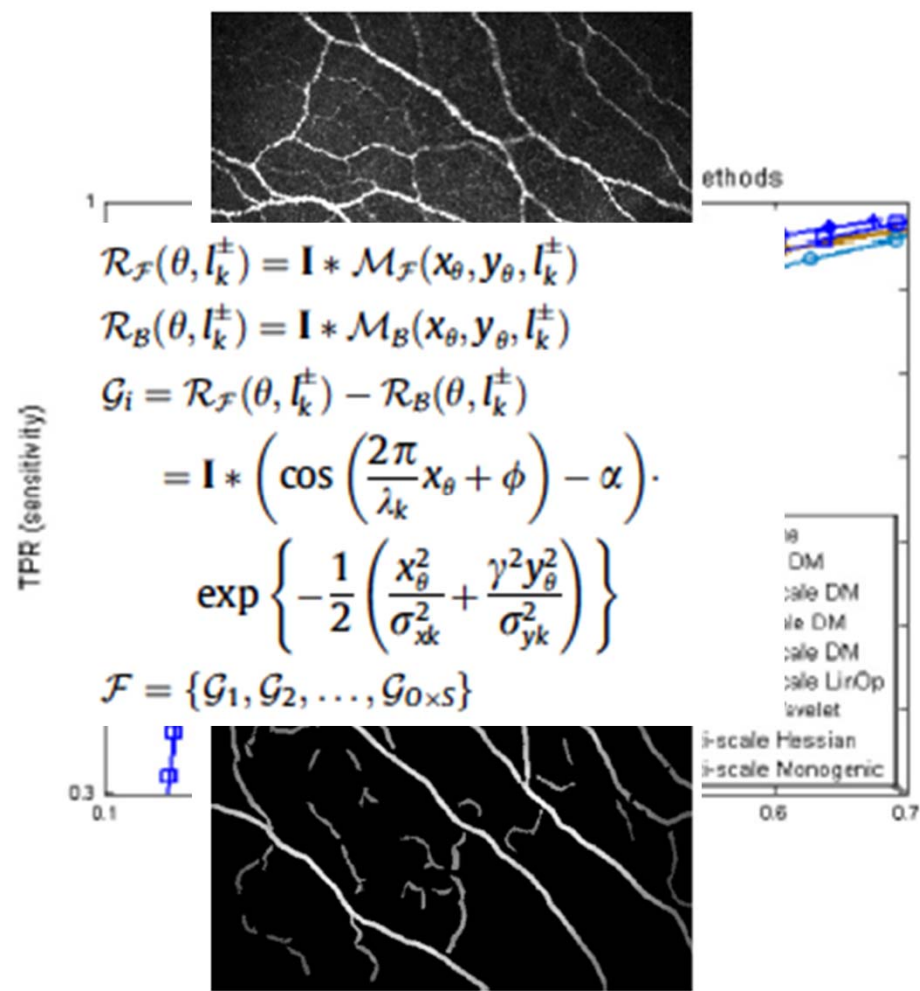
# Confocal microscopy in peripheral nerve disease

- DPN
  - Different populations
- Fabry's disease
- Idiopathic small fiber neuropathy
- CIPN
- CMT
- Autoimmune neuropathy
- Small (1-20)
- Single center
- Single reader

# Automatic analysis of diabetic peripheral neuropathy using multi-scale quantitative morphology of nerve fibres in corneal confocal microscopy imaging

M.A. Dabbah<sup>a,\*</sup>, J. Graham<sup>a,c</sup>, I.N. Petropoulos<sup>b</sup>, M. Tavakoli<sup>b</sup>, R.A. Malik<sup>b</sup>

- Saccadic eye movements are fast and can blur nerve fibers
- Nerve fibers may appear very faint due to differences of depth. The same nerve fiber could appear and disappear several times as it moves in and out of the focus plane.
- This movement can affect the visual diameter and the brightness of the fiber.



# Normative series

Age: 50 subjects

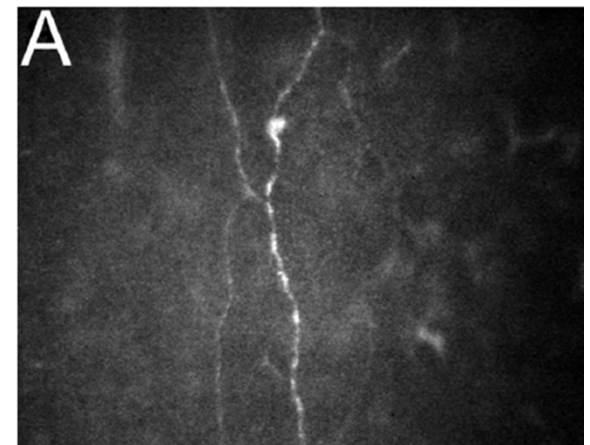
young:  $25 \pm 5$  years (median 22)

older:  $70 \pm 5$  years (median 74)

prior to cataract surgery

- After evaluation of the quality and acquisition, up to three frames including the superficial nerve plexus were archived directly to a hard disk drive. *In vivo* confocal microscopy took approximately 5 min per patient to complete. A total of 120 frames were analyzed from 50 eyes.
- Tracing of the nerves using automatic caliper tool, analySIS (3.1; Soft Imaging System, Münster, Germany).

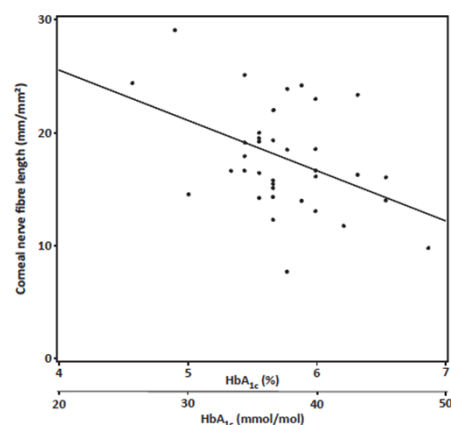
	Nerve density ( $\mu\text{m}/\text{mm}^2$ )	Nerve fibre diameter ( $\mu\text{m}$ )	Beading (/mm)
Group 1	$632.35 \pm 287.57$	$0.52 \pm 0.23$	$213 \pm 123$
Group 2	$582.39 \pm 327.13$	$0.56 \pm 0.27$	$201 \pm 192$
Statistical significance	$P < 0.005$	$P = 0.133$	$P = 0.078$



# Variables associated with corneal confocal microscopy parameters in healthy volunteers: implications for diabetic neuropathy screening

T. Wu<sup>1\*</sup>, A. Ahmed<sup>1\*</sup>, V. Bril<sup>2</sup>, A. Orszag<sup>1</sup>, E. Ng<sup>2</sup>, P. Nwe<sup>2</sup> and B. A. Perkins<sup>1</sup>

Baseline clinical characteristics	Corneal nerve fibre length (mm/mm <sup>2</sup> )		Corneal nerve fibre density (fibres/mm <sup>2</sup> )		Corneal nerve branch density (branches/mm <sup>2</sup> )		Tortuosity coefficient (unitless)	
	$\beta$	<i>P</i>	$\beta$	<i>P</i>	$\beta$	<i>P</i>	$\beta$	<i>P</i>
Univariate models*								
Age (years)	-0.07	0.04	-0.12	0.15	-0.20	0.07	0.10	0.14
Contact lens duration (years)†	0.19	0.02	0.36	0.10	0.63	0.02	-0.15	0.34
Diastolic blood pressure (mmHg)	-0.01	0.84	-0.03	0.86	-0.16	0.37	-0.23	0.03
HbA <sub>1c</sub> (mmol/mol)	-0.44	0.009	-0.62	0.15	-1.07	0.06	-0.50	0.09
HbA <sub>1c</sub> (%)	-4.90	0.009	-6.73	0.15	-11.72	0.06	-5.45	0.09
LDL cholesterol (mmol/l)	-1.65	0.11	-5.19	0.04	-3.92	0.24	-0.07	0.97



# Corneal confocal microscopy in clinical trials

- Pancreas transplant

# Corneal Confocal Microscopy Detects Early Nerve Regeneration in Diabetic Neuropathy After Simultaneous Pancreas and Kidney Transplantation

10 healthy control subjects and 15DM subjects undergoing SPK were evaluated at baseline. SPK patients were re-evaluated at 6 and 12 months

Parameter	Control subjects	Baseline	Follow-up	
			6 months	12 months
<i>n</i> (female/male)	10 (3/7)	15 (5/10)	15	15
Age (years)	47 ± 3	47 ± 3	—	—
Diabetes duration (years)	0	27 ± 3.5	—	—
BMI (kg/m <sup>2</sup> )	27 ± 1	22 ± 2	25.5 ± 1	25.5 ± 1
HbA <sub>1c</sub> (%)	5.7 ± 0.1	7.4 ± 0.8	5.9 ± 0.3	5.9 ± 0.4
Cholesterol (mmol/L)	5.1 ± 0.2	4.0 ± 0.3*	4.3 ± 0.3	4.5 ± 0.3
HDL (mmol/L)	1.5 ± 0.1	1.3 ± 0.2	1.5 ± 0.2	1.6 ± 0.2
Triglycerides (mmol/L)	1.3 ± 0.2	1.4 ± 0.1	1.2 ± 0.1	1.03 ± 0.1
Estimated glomerular filtration rate (mL/min/L)	86.22 ± 2.13	60.53 ± 8.64†	64.0 ± 7.5	66.0 ± 6.19

# Corneal Confocal Microscopy in SPK Tx

Parameter	Control subjects	Baseline	Follow-up	
			6 months	12 months
NSP (0–38)	0	6.7 ± 1.8†	7.6 ± 2.2	7.3 ± 2.0
NDS (0–10)	0.3 ± 0.2	4.6 ± 0.9†	5.0 ± 1.1	5.4 ± 0.7
McGill pain index	0	1.7 ± 0.6*	1.9 ± 0.8	1.3 ± 0.5
VPT (volts)	6.7 ± 1.8	19.4 ± 3.7*	17.4 ± 3.3	16.9 ± 3.4
CS (°C)	29.3 ± 0.4	17.5 ± 3.1†	19.8 ± 2.9	20.0 ± 2.7
WS (°C)	38.1 ± 0.8	43.7 ± 1.4†	43.8 ± 1.2	42.3 ± 1.1
Heart rate variability (average bpm)	15.3 ± 2.1	7.1 ± 1.7†	5.7 ± 1.7	4.9 ± 2.1
Sural nerve conduction velocity (m/s)	47.9 ± 0.5	40.6 ± 2.2†	41.5 ± 1.6	41.8 ± 1.9
Sural amplitude (μA)	20.7 ± 3.4	5.1 ± 0.9†	5.1 ± 0.9	4.0 ± 0.6
Peroneal nerve conduction velocity (m/s)	47.7 ± 0.9	35.9 ± 1.8‡	37.7 ± 1.2	38.5 ± 1.8
Peroneal amplitude (mV)	12.2 ± 0.9	2.4 ± 0.4‡	1.9 ± 0.4	1.7 ± 0.3

TABLE 3

Corneal sensitivity, corneal nerve morphology, and IENFD in control subjects and type 1 diabetic patients at baseline and after SPK at 6 and 12 months

Parameter	Control subjects	Baseline	Follow-up	
			6 months	12 months
NCCA (mbars)	0.56 ± 0.1	1.78 ± 0.42*	1.83 ± 0.73	1.84 ± 0.89
CNFD (no./mm <sup>2</sup> )	35.77 ± 1.53	14.44 ± 1.20‡	15.22 ± 1.63	19.27 ± 1.57*
CNBD (no./mm <sup>2</sup> )	100.92 ± 13.1	21.46 ± 3.78‡	36.85 ± 6.04*	43.02 ± 6.48†
CNFL (mm/mm <sup>2</sup> )	27.93 ± 1.26	11.35 ± 1.04‡	13.35 ± 1.50	15.63 ± 1.56*
IENFD (no./mm)	9.77 ± 1.24	2.03 ± 0.61‡	—	2.31 ± 1.17

dorsum of the foot, 2 cm above the second metatarsal head

ORIGINAL ARTICLE

**EFFECTS OF PANCREATIC TRANSPLANTATION ON DIABETIC NEUROPATHY**

WILLIAM R. KENNEDY, M.D., XAVIER NAVARRO, M.D., PH.D., FREDERICK C. GOETZ, M.D.,  
DAVID E.R. SUTHERLAND, M.D., PH.D., AND JOHN S. NAJARIAN, M.D.

(N Engl J Med 1990; 322:1031-7.)

# Long-Term Effects of Pancreatic Transplantation on Diabetic Neuropathy

Navarro X, Sutherland DER, Kennedy WR. Long-term effects of pancreatic transplantation on diabetic neuropathy. Ann Neurol 1997;42:727-736

115 transplant patients and 92 disease controls who did undergo transplants  
Followed for 10 (yes 10!) years.

# Long-Term Effects of Pancreatic Transplantation on Diabetic Neuropathy

Table 3. Neurophysiological Test Results in Diabetic Patients of the Study Group, with a Functioning PTx

	Entry	Increment with Respect to Values at Entry					
		1 Year	2 Years	3.5 Years	5 Years	7 Years	10 Years
Motor nerve (n)	(115)	(115)	(79)	(52)	(45)	(17)	(10)
Median							
NCV	$46.3 \pm 5.3$	$2.30 \pm 3.55^{b,d}$	$2.30 \pm 4.43^{b,d}$	$2.96 \pm 3.97^{b,d}$	$2.98 \pm 4.09^{b,d}$	$1.41 \pm 3.64$	$0.70 \pm 3.92^c$
MAP	$6.3 \pm 2.8$	$1.12 \pm 2.45^{b,d}$	$1.09 \pm 2.64^{b,c}$	$1.26 \pm 2.95^{b,c}$	$0.92 \pm 3.05^a$	$0.87 \pm 1.67^{a,c}$	$0.70 \pm 1.58^a$
Peroneal							
NCV	$35.8 \pm 5.9$	$1.52 \pm 3.28^{b,d}$	$2.19 \pm 3.17^{b,d}$	$3.20 \pm 4.40^{b,d}$	$3.03 \pm 5.60^{b,c}$	$2.67 \pm 5.08$	$3.22 \pm 7.96$
MAP	$1.7 \pm 2.0$	$-0.01 \pm 1.49^d$	$0.14 \pm 1.89^c$	$0.36 \pm 1.39^d$	$-0.20 \pm 1.24$	$0.24 \pm 1.68^c$	$0.57 \pm 0.96^d$
Sural							
NCV	$33.7 \pm 4.2$	$1.24 \pm 3.71$	$0.70 \pm 5.57$	$2.41 \pm 3.20^a$	$3.26 \pm 4.68$	$3.76 \pm 2.59$	$-0.94 \pm 0.00$
MAP	$1.5 \pm 2.5$	$0.56 \pm 2.75^{a,d}$	$0.92 \pm 3.79^{a,d}$	$0.76 \pm 2.30^{a,c}$	$0.36 \pm 2.35$	$1.16 \pm 2.23^{a,c}$	$1.20 \pm 2.71^c$

# Conclusions from previous pancreas transplant studies

- Our findings suggest that the progression of diabetic polyneuropathy can be halted and polyneuropathy slightly improved by successful pancreatic transplantation. However, the degree of improvement was small, probably because of previous structural damage to the peripheral nervous system. The effect of pancreatic transplantation may be greater if it is performed at an earlier stage of the disease.  
In Celiac nerves but not in other nerves suggesting that nerve less severely affected had potential to improve.

# Corneal confocal microscopy in SPK Tx

- Interpreted cautiously.
- Small study
- Short follow up
- Single center
- Only Tx patients were followed; no disease controls. ?blinding
- Not clear what the meaning is of an increase in CNFD in absence of other improvements
- Historically pancreas transplant patients' DPN improves only modestly

# Conclusions/Recommendations

- Axon loss is central to neurological disease
  - Diabetic neuropathy
  - MS
  - AD
- Measures that focus on axonal integrity and axon loss are most important in DPN. Pathology is viewed as the gold standard.
  - Nerve conduction, skin biopsy, confocal microscopy all relate to pathology

- Thank you.